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F10

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01H 5/10, 1/06

A1

(11) International Publication Number: WO 95/20313

(43) International Publication Date: 3 August 1995 (03.08.95)

(21) International Application Number: PCT/EP95/00369

(22) International Filing Date: 31 January 1995 (31.01.95)

(30) Priority Data:

9400177	31 January 1994 (31.01.94)	ES
9400178	31 January 1994 (31.01.94)	ES
9401383	24 June 1994 (24.06.94)	ES
9401384	24 June 1994 (24.06.94)	ES

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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(54) Title: SUNFLOWER SEEDS AND OIL HAVING A HIGH STEARIC ACID CONTENT

(57) Abstract

The invention relates to a sunflower seed, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds. Preferably the seeds comprise an oil having a stearic acid content of between 19.1 and 35 % by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with an alkylating agent, such as ethyl methane sulfonate in water, or with sodium azide in water. The invention further relates to sunflower oil obtainable by extracting the sunflower seeds, to a method for preparing sunflower seeds having an increased stearic acid content as compared to wild type seeds, a method for preparing a sunflower oil having an increased stearic acid content sunflower plants produced from the seeds and the use of the sunflower oil in various products.

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SUNFLOWER SEEDS AND OIL HAVING A HIGH STEARIC ACID CONTENT

The present invention relates to sunflower seeds comprising an oil having an increased stearic acid content as compared to wild type plants between 10% and 35% by weight related to the total amount of fatty acids in the oil. The invention also relates to sunflower seeds having a stearic acid content up to 54% by weight or more. The invention further relates to a sunflower oil extractable from the seeds of the invention, to sunflower plants produced from the seeds, to methods for preparing the seeds and the oil, as well as to the use of the oil in various products and to the products comprising the oil.

Sunflower is generally cultivated for obtaining oil which has saturated fatty acids (palmitic and stearic) and unsaturated fatty acids (oleic and linoleic). The stearic acid content is always less than 10% (Gustone, F.D. et al. "The lipid handbook"; Chapman and Hall 1986), normally comprised between 3% and 7%. In relation with the unsaturated fatty acids there are two different kinds of sunflower seeds: the normal sunflower which has a linoleic acid content between 50% and 70% (Knowles, P.F. "Recent advances in oil crops breeding"; AOCS Proceedings 1988) and the high oleic sunflower which has 2-10% of linoleic acid and 75-90% of oleic acid (Soldatov, K.I. "Chemical mutagenesis in sunflower breeding"; Int. Proc. 7th Intern. Sunflower Conference, 352-357, 1976). There is also a sunflower line having a high palmitic acid content, between 22% and 40% (R. Ivanov et al. "Sunflower Breeding for High Palmitic Acid Content in the Oil; Proc. of the 12th Intern. Sunflower Conference, Vol. II, 453-465, 1988) and another line with low saturated fatty acid content (6% or less) (EP-A-496504).

Table 1 shows the fatty acid composition for some known sunflower oil varieties.

Table 1

% of fatty acids in sunflower oil

Variety	Palmitic	Stearic	Oleic	Linoleic
Normal[1]	5.9	5.7	21.8	66.5
High oleic[1]	3.1	4.8	84.9	6.7
Low saturated[2]	3.9	2.2	89.9	4.0
High palmitic[3]	25.1	4.3	10.6	56.4

[1] Fernández Martínez et al.; Grasas y Aceites 37, (1986)

[2] Patent EP-A-496504

[3] This variety has also 3.6% of palmitoleic acid

The saturated fatty acid content of an oil is directly related with the physical and chemical characteristics thereof. In case that said content is sufficiently high, the oil can be a solid at room temperature like some animal fats. Normal sunflower oil is always a liquid under said conditions.

In the food industry like for the production of confectionery or margarine, animal fats or hydrogenated vegetable fats are usually used because a solid or semi-solid product is required. By means of hydrogenation unsaturated fatty acids are converted into saturated fatty acids. Animal fats as well as hydrogenated fats are not very recommendable from a nutritional point of view (Chow, C.K. "Fatty acids in food and their health implications", Dekker, N.Y., 1992). Animal fats have a relatively high cholesterol content. Too much cholesterol in the diet may be detrimental to the health. Therefore animal fats have been substituted in the last years by hydrogenated vegetable fats which do not contain cholesterol.

However, said hydrogenated fats present another problem derived from the hydrogenation process. In said process positional isomerization (shift of double bonds) and stereo-chemical transformations (formation of "trans" isomers) take place. Isomers are produced in an amount of up

to 30%-50% of the total fatty acids amount. These isomers are not very healthy from a nutritional point of view (Wood, R., "Biological effects of geometrical and positional isomers of monounsaturated fatty acids in humans"; Dekker, N.Y. (1990); Willet, W.C. & Ascherio, A., "Trans Fatty Acids: Are The Effects Only Marginal ?", American Journal of Public Health, Vol. 84, 5, (1994)). Therefore, the use of hydrogenated fats in the food industry should be avoided.

Sunflower oil has a desirable content of unsaturated fatty acids. For use in the food industry however, the stearic acid content of the oil must be higher than in the normal sunflower oil (Norris, M.E., "Oil substitutions in food formulations", Inform. 1, 388-392 (1990)) in order to obtain a more solid product.

It is thus an object of the invention to provide a new natural vegetable oil extracted from mutated seeds, the oil having a higher stearic acid content as compared to oil obtained from wild type seeds.

The invention therefore provides sunflower seeds, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds.

Preferably the sunflower seeds according to the invention comprise an oil having a stearic acid content of between 19.1 and 35% by weight, related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds during 2 hours at room temperature with an alkylating agent such as a solution of 70 mM ethyl methane sulfonate in water.

In another embodiment of the invention the seeds comprise an oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with a solution of 2 mM sodium azide in water during 2 hours at room temperature.

Sunflower seeds identified as "CAS-3" having an average stearic acid content of 25% by weight, related to the total amount of fatty acids in the oil, have been deposited on December 14, 1994 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, U.S.A. under deposit accession number ATCC 75968. Sunflower seeds identified as "CAS-4" having an average stearic acid content of 15.4% by weight, related to the total amount of fatty acids in the oil, have been deposited on the same day with the same institution under deposit accession number ATCC 75969.

Seeds having an even higher stearic acid content between 29 and 54% by weight related to the total amount of fatty acids in the oil, may be obtained according to the invention by crossing sunflowers originating from seeds having a stearic acid content between 19.1 and 35% by weight with sunflowers originating from seeds having a stearic acid content between 10 and 19% by weight, and collecting the seeds.

The invention further relates to sunflower oil having a stearic acid content of between 10 and 54% by weight, preferably between 10 and 35% by weight, related to the total amount of fatty acids in the oil, which may be obtained by extracting sunflower seeds of the invention. Sunflower oil having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, may be obtained by extracting sunflower seeds having the deposit accession number ATCC 75969. Sunflower oil having a stearic acid content of 25% by weight related to the total amount of fatty acids in the oil, is obtainable by extracting sunflower seeds having the deposit accession number ATCC 75968.

CLAIMS

1. Sunflower seed, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds.

2. Sunflower seed as claimed in claim 1, **characterized in that** the seeds comprise an oil having a stearic acid content of between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with an alkylating agent.

3. Sunflower seeds as claimed in claim 2, **characterized in that** the parent seeds are treated during 2 hours at room temperature with a solution of 70 mM ethyl methane sulfonate in water.

4. Sunflower seed as claimed in claim 1, **characterized in that** the seeds comprise an oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with a solution of 2 mM sodium azide in water.

5. Sunflower seed having a stearic acid content of 25% by weight related to the total amount of fatty acids in the oil, obtainable from the American Type Culture Collection under deposit accession number ATCC 75968.

6. Sunflower seed having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, obtainable from the American Type Culture Collection under deposit accession number ATCC 75969.

CLAIMS

seed, comprising a sunflower oil
earic acid content as compared to wild
by treating parent seeds with a
a period of time and in a
nt to induce one or more mutations in
lved in stearic acid biosynthesis
sed production of stearic acid,
d seeds and culturing progeny plants
and analyzing progeny seeds, selecting
ed the desirable genetic trait and
ıe cycle of germination, culturing and

seed as claimed in claim 1,
:he seeds comprise an oil having a
: between 19.1 and 35% by weight
ıount of fatty acids in the oil, and
:ing the parent seeds with an

seeds as claimed in claim 2,
he parent seeds are treated during 2
re with a solution of 70 mM ethyl
ter.
seed as claimed in claim 1,
he seeds comprise an oil having a
between 10 and 19% by weight related
fatty acids in the oil, and are
the parent seeds with a solution of 2
r.
seed having a stearic acid content of
ɔ the total amount of fatty acids in
n the American Type Culture
: accession number ATCC 75968.
seed having a stearic acid content of
to the total amount of fatty acids
:rom the American Type Culture
: accession number ATCC 75969.

/ing a stearic acid content
elated to the total amount of
ıble by crossing sunflowers
ıg to claims 2, 3 and 5 with
eds according to claims 4 and

/ing a stearic acid content as
ıs 1-7, and in addition a
and 40% by weight or an
ıd 85% by weight or a linoleic
by weight, all related to the
the oil, or any combination
ıcid contents, obtainable by
ɔm the mutant seeds according
'ing a desired phenotype with
ınt.
ıng a stearic acid content
lated to the total amount of
ble by extracting sunflower
d 4.
ing a stearic acid content of
total amount of fatty acids
cting sunflower seeds as

ing a stearic acid content
related to the total amount
inable by extracting
laims 1, 2 and 3.
ing a stearic acid content of
tal amount of fatty acids in
ıg sunflower seeds as claimed

ing a stearic acid content
lated to the total amount of
ɔle by extracting sunflower

:laimed in any one of the
ılmitic acid content between

7. Sunflower seed having a stearic acid content between 29 and 54% by weight related to the total amount of fatty acids in the oil, obtainable by crossing sunflowers originating from seeds according to claims 2, 3 and 5 with sunflowers originating from seeds according to claims 4 and 6, and collecting the seeds.

8. Sunflower seed having a stearic acid content as claimed in any one of the claims 1-7, and in addition a palmitic acid content between 3 and 40% by weight or an oleic acid content between 3 and 85% by weight or a linoleic acid content between 2 and 84% by weight, all related to the total amount of fatty acids in the oil, or any combination of one or more of these fatty acid contents, obtainable by crossing plants originating from the mutant seeds according to claims 1-7 with a plant showing a desired phenotype with respect to its fatty acid content.

9. Sunflower oil having a stearic acid content between 10 and 19% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claims 1 and 4.

10. Sunflower oil having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 6.

11. Sunflower oil having a stearic acid content between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claims 1, 2 and 3.

12. Sunflower oil having a stearic acid content of 25% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 5.

13. Sunflower oil having a stearic acid content between 29 and 54% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 7.

14. Sunflower oil as claimed in any one of the claims 9-13, further having a palmitic acid content between

3 and 40% by weight, an oleic acid content between 3 and 85% by weight and a linoleic acid content between 2 and 84% by weight, all related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 8.

15. Method for preparing sunflower seeds having an increased stearic acid content as compared to wild type seeds, by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the parent seeds, culturing progeny plants from the parent seeds, collecting progeny seeds and optionally repeating the cycle of germination, culturing and collection of seeds.

16. Method as claimed in claim 15, **characterized in that** the parent seeds are treated during 2 hours at room temperature with a solution of an alkylating agent, such as 70 mM ethyl methane sulfonate in water.

17. Method as claimed in claim 15, **characterized in that** the parent seeds are treated during 2 hours at room temperature with a solution of 2 mM sodium azide in water.

18. Method for preparing a sunflower oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claims 1 and 4.

19. Method for preparing a sunflower oil having a stearic acid content of between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claims 1, 2 and 3.

20. Method for preparing a sunflower oil having a stearic acid content of between 29 and 54% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claim 7.

21. Sunflower plant produced from seeds as claimed in any one of the claims 1-8.

22. Use of a sunflower oil as claimed in claims 9-14 in the production of edible fats or fat mixtures, such as margarine or vegetable-dairy.

23. Use of a sunflower oil as claimed in claims 9-14 in confectionery or bakery.

24. Margarine comprising a sunflower oil as claimed in any one of the claims 9-14.

25. Vegetable-dairy comprising a sunflower oil as claimed in any one of the claims 9-14.

26. Confectionery comprising a sunflower oil as claimed in any one of the claims 9-14.

27. Bakery comprising a sunflower oil as claimed in any one of the claims 9-14.